

VIBRATING NANONEEDLE AND LAB-ON-CHIP MICROFLUIDICS SYSTEM
FOR SINGLE CELL MECHANICS

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VIBRATING NANONEEDLE AND LAB-ON-CHIP MICROFLUIDICS SYSTEM
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Specially dedicated to my beloved parents and lovely wife Nusrat Jahan.

Also for those who want to be researchers.

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ABSTRACT

Single cell mechanics is a vital part of single cell analysis. It has attracted great interest among scientists as cell mechanics can be linked to early diagnosis of diseases. To date, several great findings have been achieved in the study of single cell mechanics. Nevertheless, more work are required to enable the technology to be pushed to the frontier of single cell mechanics. Considering this objective, this work focuses on the technological development of two major parameters of single cell mechanics: Single Cell Wall (SCW) cutting operations (Phase 01) and Single Cell Mass (SCM) measurement (Phase 02). A *saccharomyces cerevisiae* yeast cell was used as a sample cell. In phase 01, a vibrating nanoneedle (tungsten) integrated with lead zirconate titanate piezoelectric actuator was used for SCW cutting operation. Two different frequencies of vibrating nanoneedle were used for cell wall cutting operation: 1 Hz and 10 Hz. For a constant penetration depth of 1.2 μm , the obtained cell nanoneedle's velocities were 7 $\mu\text{m/s}$ and 24 $\mu\text{m/s}$. Results show that faster nanoneedle causes less damage to the cell surface. In phase 02, a Lab-On-Chip microfluidics system was used for SCM measurement. SCM result was extracted from the relation between drag force applied on cell and Newton's law of motion. Drag force on the cell has been generated by a pressure driven syringe micropump. This approach of measuring SCM was calibrated using a known mass (73.5 pico gram) of polystyrene particle of 5.2 μm diameter. Different sizes (2-7 μm diameter) of yeast cells were cultured in our laboratory. Mass of 4.4 μm diameter of yeast cell was measured as 2.12 pg. In addition, results show that single yeast cell mass increases exponentially with the increase of cell size. It is envisaged that this work i.e. combination of single cell cutting operation and single cell mass measurement system will add a significant contribution to the knowledge of cell mechanics and single cell analysis.

ABSTRAK

Mekanik sel tunggal adalah penting dalam analisis sel tunggal. Ia telah menarik minat yang tinggi di kalangan ahli sains kerana mekanik sel boleh dikaitkan dengan diagnosis awal penyakit. Setakat ini, beberapa penemuan besar telah dicapai dalam mengkaji mekanik sel tunggal. Walau bagaimanapun, banyak perkara perlu dilakukan untuk membolehkan teknologi ini dibawa ke tahap paling tinggi dalam analisis mekanik sel tunggal. Bagi mencapai objektif ini, tumpuan diberikan kepada pembangunan teknologi bagi dua parameter utama mekanik sel iaitu operasi memotong Dinding Sel Tunggal (SCW) (Fasa 01) dan pengukuran Jisim Sel Tunggal (SCM) (Fasa 02). Sel yis *saccharomyces cerevisiae* digunakan sebagai sel sampel. Dalam fasa 01, jarum nano bergetar (tungsten) disepadukan dengan penggerak piezoelektrik pelambam zirkonat titanat telah digunakan untuk operasi memotong SCW. Dua frekuensi jarum nano bergetar yang berbeza telah digunakan untuk operasi pemotongan dinding sel: 1 Hz dan 10 Hz. Bagi mendapat kedalaman penembusan berterusan sebanyak 1.2 μm , halaju penembusan dinding sel yang didapati adalah sebanyak 7 $\mu\text{m/s}$ dan 24 $\mu\text{m/s}$. Keputusan menunjukkan bahawa jarum nano yang lebih laju dapat mengurangkan kerosakan pada permukaan sel. Dalam fasa 02, satu sistem mikrobendalir *Lab-On-Chip* telah digunakan bagi pengukuran SCM. Jisim sel tunggal dikira melalui daya seretan yang dikenakan kepada sel yang dikaitkan dengan hukum gerakan Newton. Kuasa seretan pada sel telah dijanakan oleh picagari pam mikro dipandukan tekanan. Sistem ini dikalibrasi menggunakan jisim zarah polistirena (73.5 pico gram) berdiameter 5.2 μm . Sel yis bagi saiz yang berbeza (2-7 μm diameter) telah dikultur di makmal. Jisim sel yis tunggal bagi saiz diameter 4.4 μm telah diukur sebagai 2.12 pg. Di samping itu, keputusan menunjukkan bahawa jisim sel yis tunggal mengalami peningkatan secara eksponens dengan peningkatan saiz sel. Adalah dijangka bahawa kerja ini iaitu gabungan pemotongan sel dan sistem pengukuran jisim sel tunggal akan menambah nilai yang besar terhadap bidang mekanik sel dan analisis sel tunggal.

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LIST OF ABBREVIATIONS

SCA	-	Single Cell Analysis
ESEM	-	Environmental Scanning Electron Microscope
MEMS	-	Micro Electro Mechanical Systems
LOC	-	Lab-On-Chip
SCW	-	Single Cell Wall
SCM	-	Single Cell Mass
SMR	-	Suspended Microchannel Resonator
LCA	-	Living Cantilever Arrays
PMMS	-	Pedestal Mass Measurement System
PZT	-	Lead Zirconate Titanate
PDMS	-	Polydimethylsiloxane
FE	-	Finite Element
TEM	-	Transmission Electronmicroscope
SEM	-	Scanning Electronmicroscope
HV	-	High Vacuum
FIB	-	Focused Ion Beam
SMR	-	Suspended Microchannel Resonator
PSD	-	Position Sensitive Photodetector
RIE	-	Reactive Ion Etching
LDV	-	Laser Doppler Vibrometer
DEP	-	Dielectrophoresis
DOF	-	Degree Of Freedom
PFA	-	Paraformaldehyde

LIST OF SYMBOLS

F	-	Force
K	-	Spring Constant
Φ	-	Displacement Angle
L	-	Length
f	-	Resonant Frequency
m^*	-	Effective Mass
m	-	Mass
A	-	Area
$\Delta\sigma$	-	Surface Mass Loading
E	-	Elastic Modulus
b	-	Width
T	-	Thickness
F_d	-	Drag Force
P	-	Density
V	-	Cell Velocity
Re	-	Reynolds Number
a	-	Acceleration

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CHAPTER 1

INTRODUCTION

1.1 Background of the Research

The fundamental structure of a living organism is cell. Millions of cells are combined together to develop a total structure of tissue. Therefore, single cell analysis plays a significant role in tissue engineering. Conventional medical science researches are based on a population cell analysis that are derived from an average data. However, the average data is not able to illustrate the basic physiological properties of cell such as cell membrane stiffness, cell wall thickness at different cell growth, cell proliferations etc. [1]. For instance, abnormal cell growth causes cancer or tumor [2-3] by which intracellular and extracellular mechanical properties change significantly [4-5]. From the biochemical experiments it might be possible to identify that the cell growth is abnormal, but to identify the exact changes in intracellular properties, it is necessary to analyze cell's mechanical property individually. This is why we are focusing on single cell analysis (SCA). With the revolution of micro-bio and nano-bio technologies, physiology of single cell is being explored day by day. Great strides have been taken to develop the technology to investigate the intracellular and extracellular properties of single cell. For example analysis of single cell inside environmental scanning electron microscope (ESEM) [1], [6]–[8], AFM cantilever for single cell strength analysis [9], nanoscale electrochemical probe for single cell analysis (SCA) [10], SCA through electrochemical detection [6], [10]–[15] and microfluidics disk for single cell viability detection [16]. In general, single cell analysis can be divided into four categories (Figure 1.1).

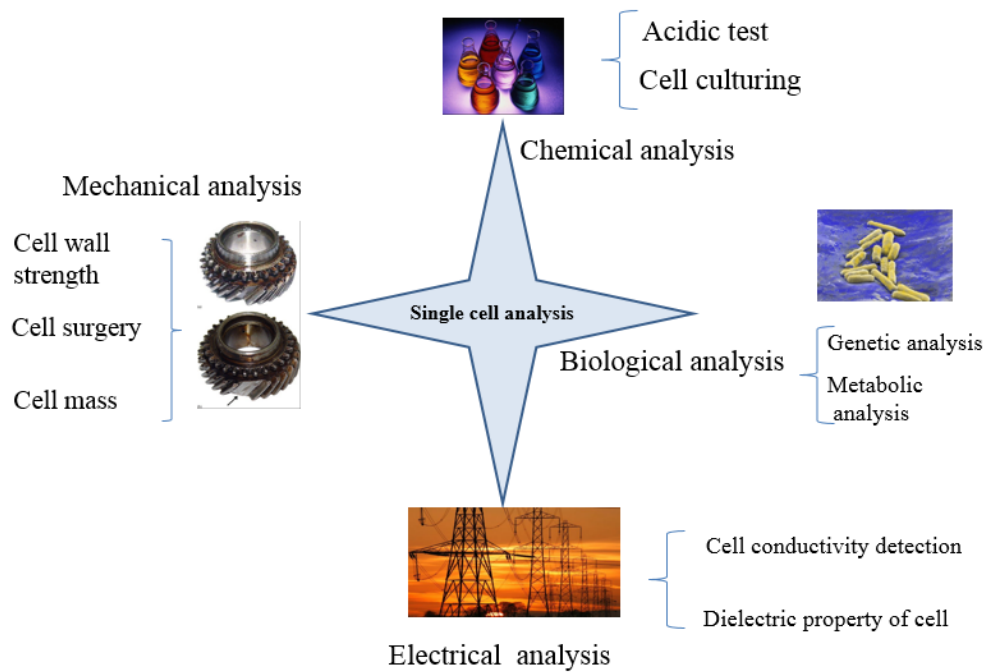


Figure 1.1: Four major branches of single cell analysis: chemical analysis; biological analysis; electrical analysis and mechanical analysis.

These are single cell's biological analysis [17]; single cell's chemical analysis [17], [18]; single cell's electrical properties analysis [19]; single cell's mechanical properties analysis [20], [21]. Among these four branches single cell, mechanical property (or cell mechanics) is an important branch of SCA. It elucidates the complex intra cellular properties of cell like cell wall strength, cell mass, cell density, cell adhesion force, cell stiffness etc. In this work, we are focusing on the sensor development for single cell wall cutting operation and single cell mass measurement.

1.2 Applications of Cell Mechanics

Recent development of micro electro mechanical systems (MEMS) provide an excellent platform to study cell mechanics, often known as lab-on-chip (LOC) microfluidics device [12], [15], [17]. Cell mechanics consist of (but not limited to) cell wall cutting operation, cell mass, density, cell stiffness, cell adhesion force and cell's viscoelastic properties etc. Chronic diseases like cancer, tumour affect the intracellular

properties of cells [26], eventually lead to change of cell mechanics [28-29]. For example, in a tumour infected cell, integrity of DNA faces continuous challenges and genomic instability occurs to the chromosome's structure [29]. Inevitably, this will cause severe change to DNA replication, cytoplasm density and cell volume which ultimately leads to the changes in single cell mass and cell wall strength. Figure 1.2 depicts this concept. When a cell becomes infected its physiological properties change and propagate to others. At a certain stage, it causes disease and requires further treatment. In this condition, before propagating to the other cells, if it is possible to identify the particular infected cell based on the cell's mechanics, then physicians will be able to diagnose the disease in a much earlier stage. Currently, scientists are using cell mechanics to diagnose disease such as:

- Hematologic disease like dengue, malaria diagnosis using cell mechanics [30], [31].
- Cell mechanics for cancer cell separation [32].
- Tumor cell detection using cell mechanics [33].

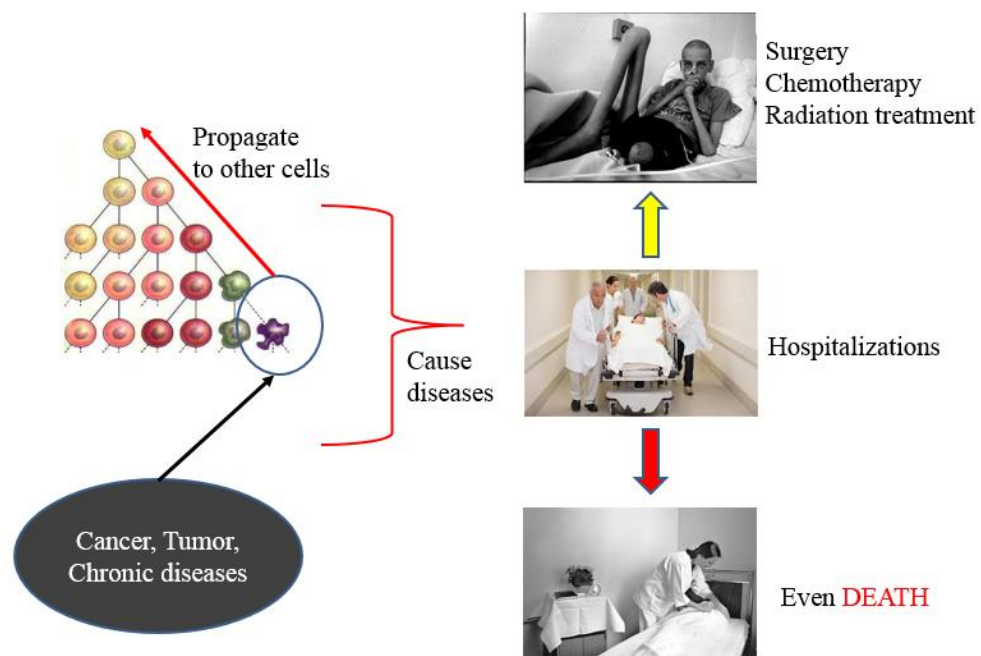


Figure 1.2: Chronic diseases infect intracellular property and propagate to others cells. Ultimately, lead to the severe diseases and death.

1.3 Statement of the Problem

Since decades, researcher are developing sensors or technologies to study single cell mechanics. Cell mechanics consist of (but not limited to) cell wall cutting operation, cell mass, cell density, cell stiffness, cell adhesion force, cell's viscoelastic properties etc. However, in this work, we are focusing on the two major issues of cell mechanics; single cell cutting operation (SCW) and the single cell mass (SCM) measurement.

a) First Issue: Single cell wall (SCW) Cutting Operation

One of the burning questions of scientist is how strong the cell wall and how much force requires to perform cell wall cutting. To realize this issue several sensors have been developed so far. For example; diamond and glass knives were used for ultrathin cryosectioning of cells [35-36]. Due to the sturdy edge of diamond knife and high edge angle (40° to 60°), it generates a very high compression stress on the upper surface of cells which may damage the cell structure. Recently, our colleagues Yajing Shen *et al.* fabricated a novel nanoknife by focused ion beam (FIB) etching of a commercial atomic force microscopy (AFM) cantilever [36] to perform cell cutting inside environmental scanning electron microscope (ESEM). However, both of the works were limited to single cell slice generation only. The reported data is not adequate to explain the strength of the single cell wall. The mechanical properties of the cell wall are partially extracted and yet under the area of “near total darkness” [6]. For instance, strength of the cell wall, cell wall thickness growth pattern in different phases of cell growth, further more molecular stricter of single cell wall. In order to bring out technological advancement for cell wall studies, this study focuses on single cell wall cutting operations also known as cell surgery specifically.

b) Second Issue : Single cell mass (SCM) Measurement

Another important parameter of cell mechanics is cell mass. Cell mass depends on the synthesis of proteins, DNA replication, cell wall stiffness, cell cytoplasm density, cell growth, ribosome and other analogous of organisms [37]. As a result, it becomes a great interest of scientists to characterize single cell mass. Lab-on-chip

microfluidics system provides an excellent platform to measure single cell mass. For example: Suspended microchannel resonator (SMR) for dry cell mass measurement, living cantilever arrays (LCA) for live cell mass measurement, Pedestal mass measurement system (PMMS) for adherent cell mass measurement. However, current technological advancements of cell mass measurement require complex fabrication procedures and the tedious experimental steps [38]. But this work focuses on a simple microfluidic system development where single cell mass can be measured from single cell flow and drag force exerted on the cell surface to generate the flow. It is envisaged that, this approach can be useful for rapid measurement of single cell mass and it may lead us to the solution of further questions on cell mechanics.

Moreover, by consolidating these two approaches of cell mechanics, intrinsic property of single cell will be elucidated. Perhaps, it may provide new tools for disease diagnosis through the variation of single cell's intrinsic property of identical cells at different health conditions.

1.4 Objectives of the Research

The objective of the research is to resolve the two aforementioned major issues of cell mechanics. The first objective of this work is to propose a novel method for single cell wall (SCW) cutting operation, which is a piezoelectric-actuated vibrating rigid nanoneedle for SCW cutting operation. The second objective of this work is to develop lab-on-chip microfluidics system for single cell mass (SCM) measurement, where rapid measurement of SCM can be performed using drag force inside microfluidic channel.

1.5 Scopes of the Research

- 1) Single cell wall cutting operations was carried out using finite element software ABAQUS 6.12 CAE/CEL and the sensor has been calibrated experimentally.
- 2) Piezoelectric actuator was used to vibrate the nanoneedle for single cell wall cutting. Inverse piezoelectric effect was used to actuate the nanoneedle.
- 3) Polydimethylsiloxane (PDMS) material has been used to fabricate the LOC microfluidics system. PDMS is a transparent, biocompatible material and sample can be observed directly under inverted microscopy.
- 4) *Saccharomyces cerevisiae* type of yeast cell has been used as a sample cell for cell wall cutting operations and cell mass measurement.

1.6 Flow of the Research

Research activities have been carried out in two phases. The first phase (Phase 01) focuses on the first issue i.e. single cell wall (SCW) cutting operation and the second phase (Phase 02) focuses on the second issue i.e. single cell mass (SCM) measurement. Figure 1.3 illustrates the flow of the research activities. Each phase of the work started with literature review followed by proposed idea, design and fabrication, calibrations and results analysis. Both SCW cutting operations and SCW measurement under the same umbrella of single cell mechanics. This thesis is the combination of these aforementioned phases reflecting single cell mechanics in terms of SCW cutting operations and SCM measurement method.

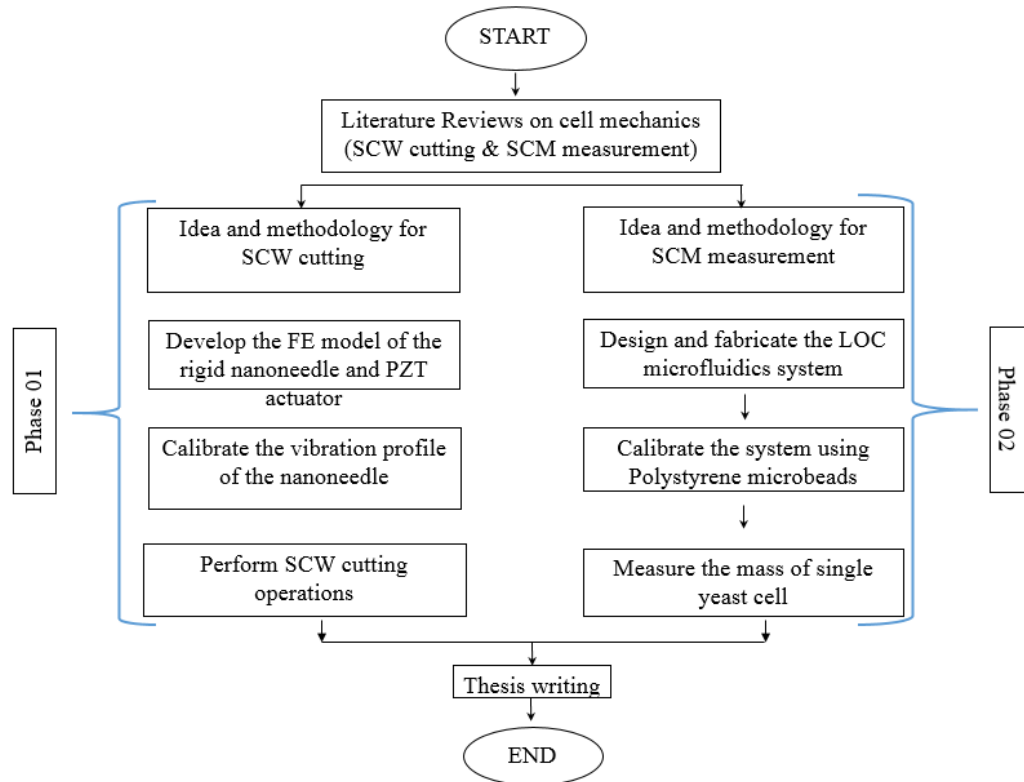


Figure 1.3: Flow of the research work. Entire work is divided into two phases. Phase 01 describes SCW cutting operations and the Phase 02 describes SCM measurement.

1.7 Organization of the Thesis

This thesis has been divided into six chapters. This chapter highlights the background of single cell analysis, importance of cell mechanics, problem statement of the research, objectives and scopes of the research and also brief summary of the research flow. The research objectives has been divided in two phases; phase 01: Single Cell Wall (SCW) cutting operations, phase 02: Single Cell Mass (SCM) measurement.

Chapter 2 presents literature review of cell mechanics, cell surgery and single cell mass measurement. Summary of the works were n presented in table and tree diagram.

Chapter 3 describes the methodology of the two phases of works. First section illustrated the proposed method for single cell cutting operations. It also described the assembling of the nanoneedle with the PZT actuator. FE model of nanoneedle and PZT also been showed in this section. In the second section, design of the proposed microfluidics chip for single cell mass measurement was presented. Theory behind SCM using drag force and Newton law of motion was also been presented in this section.

Chapter 4 illustrates the calibration of the devices. Vibration of the nanoneedle was controlled by applying voltage to the PZT actuator. Displacement of 4.5 μm was obtained from an applied voltage of 150 V. Calibration of the LOC microfluidics system was also been presented in this chapter. Microfluidics system was calibrated using a known mass of polystyrene microbeads.

Chapter 5 presents the results of phase 01 and phase 02 i.e. single cell wall cutting operations and single cell mass measurement respectively. *Saccharomyces cerevisiae* yeast cell was used as a sample cell. Effect of the nanoneedle's vibration frequency to the cell wall cutting; effect of the nanoneedle tip edge angle and the effect flat tip cylindrical nanoneedle were discussed in the first section of this chapter. While at the second section, single yeast cell mass measurement was reported. Different sizes of yeast cells (2.5 μm , 3.5 μm , 5.5 μm) were cultured to measure single cell mass.

Finally, Chapter 6 presents the conclusions of the entire work with a brief directions of the future works.

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